

References

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DIA-BLV -Ab

Enzyme immunoassay for the detection of antibodies against Bovine leukemia virus (BLV) in cattle serum

192 tests

Product code: T-10106

EXAMPLE FORM

INTENDED USE

The test kit is intended for screening of bovine serum for the presence of antibodies to *Bovine leukaemia virus* by ELISA.

INTRODUCTION

Bovine leucosis is chronic lymphoproliferative infectious disease caused by retrovirus affecting B-lymphocytes. Bovine leukaemia virus (BLV) initiates epigenomic failures in processes of maturation and differentiation of cells of haematogenic organs, in particular, in association with different components of connective tissues that later on causes lymphosarcoma. An asymptomatic course of disease sometimes comes to pass.

According to recommendation of International Epizootic Bureau during screening of bovine blood samples for BLV ELISA method are applied that detects specific IgG antibodies to BLV. The high sensitivity and specificity of the method allow to investigate large number of serum samples without their prior treatment before assay performance. Usually recombinant proteins p24 (core) and gp51 (envelope) that are analogues of specific proteins of BLV are used as antigens for coating in those kits.

PRINCIPLE OF PROCEDURE

The main kit components are microelisa strips and the conjugate. The microelisa plate is a polysterene plate coated with a mixture of recombinant proteins p24 and gp51 – analogues of specific Bovine leukaemia virus (BLV) antigens. The conjugate is represented by monoclonal antibodies to bovine IgG conjugated with a horseradish peroxidase.

When investigated specimen of bovine serum is placed into wells, antibodies that are specific to BLV bind to antigens on the solid phase, forming antigen-antibody

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complexes. Formed complexes are detected with the help of specific immunoenzyme conjugate. Non-bound components are to be washed out. Following washing procedure, a developer solution containing enzyme substrate and chromogen (tetramethylbenzidine, TMB) are added to wells. The reaction is stopped by a stop-reagent. The optical density (OD) values in wells is determined at 450 nm, this values being correlated with specific antibody concentration in investigated serum samples.

STORAGE CONDITIONS AND TRANSPORTATION

The kit must be stored and transported at 2-8°C. The kit is not subject to freezing. The shelf life of the kit is 12 months.

KIT REAGENTS

For *in vitro* diagnostic use.

Each kit contains:

N	Reagents	Presentation
1	Stock washing solution (№1) (concentrate 45X) Phosphate buffer, containing 2.2 % Triton X100.	3 bottles 3 × 25 ml
2	Microelisa strips 6 strips per plate each with 16 wells coated with a mixture of recombinant proteins p24 and gp51 – analogues of specific Bovine leukaemia virus (BLV) antigens.	2 plates (12 x 16)
3	Solution for sera dilution (№3) Phosphate buffer, containing skimmed powdered milk. Preservatives: 0.01% thimerosal.	1 bottle 1 × 20 ml
4	Solution for conjugate dilution (№4) Phosphate buffer, containing skimmed powdered milk. Preservatives: 0.01% thimerosal.	1 bottle 1 × 26 ml
5	Solution for chromogen preparation (№ 5T) Citrate-phosphate buffer, containing 0.016 % hydrogen peroxide.	1 bottle 1 × 14 ml
6	Chromogen (TMB) Solution containing 0.03% 3,3',5,5'-tetramethylbenzidine.	1 bottle 1 × 14 ml
7	Positive control (C+) Cattle serum containing antibodies to Bovine leukaemia virus. Preservatives: 0.1% sodium azide and 0.01% paranitrophenol.	1 vial 1 × 0.15 ml

a blank well; for that purpose include an empty well in the run or at 450/620 nm using a dual wavelength (this way is more preferable).

Reading and interpretation results

NC – absorbance of the negative control

PC – absorbance of the positive control

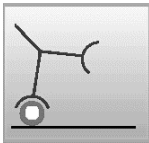
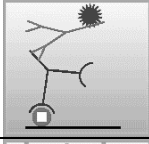
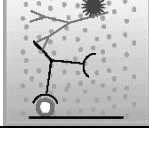
\overline{NC} – mean absorbance of the negative control

- The mean absorbance value of negative controls (\overline{NC}) is calculated.
- The assay performance is considered as valid if \overline{NC} is not higher than 0.200.
- If one of two NC is higher than 0.200 such value is excluded then calculation is run using rest NC value that is below 0.200.
- The assay performance is considered as valid if PC of each well with the positive control is not less than 0.600.
- Calculate **Cut-off** value: $\text{Cut-off} = \overline{NC} + 0.12$.
- Determine the grey zone.

The grey zone is the zone with absorbance value within the range:
 $\text{Cut-off} - 10\% \leq OD \leq \text{Cut-off}$.

- The result is considered **negative** if the optical density is below the grey zone.
- The result is considered **positive** if the optical density is equal or greater than the grey zone.
- Specimens with absorbance value lying within the grey zone range are considered **indeterminate** and should be retested in duplicate.
 - specimens positive in one or more wells are interpreted as positive ones;
 - specimens negative in both wells are interpreted as negative ones.

OVERVIEW OF PROCEDURE

<ul style="list-style-type: none"> • Dispense 95 µl of specimen diluent and 5 µl both controls and investigated serum samples in wells • Incubate for 60 min at 37°C (forming complex antigen-antibody) • Wash 4 times with washing solution 	
<ul style="list-style-type: none"> • Dispense 100 µl of conjugate solution in wells • Incubate for 30 min at 37°C (forming complex antigen-antibody-HRP) • Wash 6 times with washing solution 	
<ul style="list-style-type: none"> • Dispense 100 µl TMB substrate in wells • Incubate for 30 min at room temperature (colouring) • Stop the reaction by adding 100 µl stop-reagent • Read measuring the optical density at 450/620 nm 	

- Prevent the direct light to fall on the working surface during ELISA procedure.
- Use a new tip for bringing specimens into wells.
- Never use the same trough for distribution conjugate and TMB substrate.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay procedure.

Wash procedure

Washing is to be strictly performed according to the instructions, as insufficient plate washing leads to incorrect results.

Use automatic washer*, as recommended; in case of its absence or faulty work – use multi-channel pipette for washing.

Follow this procedure in each washing:

- aspirate the wells contents completely into a waste flask;
- then fill the wells completely with washing solution (control the filling of wells, the dispensed volume is to be not less than 350 µl per well) avoiding overflow of buffer from one well to another;
- aspirate completely.

Make sure that no fluid remains on the top and the bottom of the strips and stripholder after the last aspiration (e. g. by blotting with absorbent tissue).

* Contact our company for further information on the different types of washers validated by our technical services.

Test procedure

- Fit the stripholder with required number of **strips**.
- Wash strips with washing solution once (according the section *Wash procedure*).
- Pipette 95 µl of the **solution for sera dilution (№3)** into each well.
- Distribute in the wells the following components:
 - wells: A1, B1 - 5 µl of **positive control (C+)**.
 - wells: C1, D1, - 5 µl of **negative control (C-)**.
 - the rest wells : 5 µl of **specimens**.

Carefully repipette mixture in wells.

- Cover the plate with adhesive film and incubate at 37°C for 60 minutes.
- Aspirate the contents of all wells and wash the plate with **washing solution** 4 times (according the section *Wash procedure*). If necessary, dry the plate by slight tapping upside-down on absorbent paper.
- Put 100 µl of the **conjugate solution** into each well.
- Cover the plate with adhesive film and incubate at 37°C for 30 minutes.
- Aspirate the contents of all wells and wash the plate with **washing solution** 6 times (according the section *Wash procedure*). If necessary, dry the plate by slight tapping upside-down on absorbent paper.
- Pipette into each well 100 µl of the **TMB substrate**.
- Cover the plate with adhesive film and incubate at room temperature (18-25°C) for 30 minutes in the darkness.
- Add 100 µl of **stop-reagent** into each well to stop colour reaction (maintain the same pipetting sequence and rate used for TMB substrate addition).
- Treatment of results is conducted with reader within 5 min after reaction stopping. The optical density is defined with the reader at 450 nm (single wavelength) against

N	Reagents	Presentation
8	Negative control (C-) Cattle negative serum. Preservatives: 0.1% sodium azide and 0.01% paranitrophenol.	1 vial 1 × 0.15 ml
9	Conjugate (concentrate 50X) Monoclonal antibodies against cattle IgG bound to a horseradish peroxidase (HRP). Preservatives: 0.01% thimerosal.	1 vial 1 × 0.6 ml
10	Stop-reagent 0.5 M sulphuric acid solution.	1 bottle 1 × 25 ml
11	Adhesive film	6 items

ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED

- distilled or deionized water;
- disposable gloves;
- disposable V-shaped troughs;
- vials for reagent preparation (glass or plastic);
- graduated cylinder (1000 ml);
- absorbent paper;
- hydrogen peroxide 6% or other accepted disinfectant;
- sodium bicarbonate;
- ethanol, 70°;
- automatic single-channel pipettes (e.g. 5-40, 20-200, 200-1000 µl) with disposable tips;
- automatic multi-channel pipettes (50-300 µl) with disposable tips;
- incubator, 37±1°C;
- microwell wash system*;
- microwell reader* (with dual wavelength 450/620);
- biohazard waste containers for potentially contaminated materials.

* Contact our company for further information on the equipment validated by our technical services.

SAFETY PRECAUTIONS AND WARNINGS

- Use a new tip for pipetting specimens into wells.
- All reagents included in the kit are intended for "*in vitro*" diagnostic use.
- Wear disposable gloves when handling reagents and samples and thoroughly wash hands after handling them.
- Do not pipette by mouth.
- All liquid biohazardous wastes are to be treated by hydrogen peroxide 6% at room temperature during 3 hours.
- All solid wastes are to be stored in a special container and autoclaved during 1 hour at 120°C.
- Instruments, equipment and also working surfaces are to be treated by with 70° ethanol.

SPECIMEN PREPARATION

Serum specimens are to be stored at 2-8°C for 72 hours. If necessary these specimens may be frozen (more than two freezing-thawing procedures are not allowed) at temperature below -20°C.

All specimens containing aggregates and visible suspended particles are to be clarified by centrifugation.

Specimens with sodium azide, hemolysis, hyperlipidemiae or bacterial contamination may not be used in the ELISA procedure.

ASSAY PROCEDURE

Reagents and specimens should be at room temperature (18-25°C) before beginning the assay and can be remained at room temperature during testing. After use return reagents to 2-8°C.

Reagents preparation

Microelisa strips

Open the pack and remove the plate. Return unused strips in the pack. Reseal the pack and return to 2-8°C.

The strips are stable for 4 weeks at 2-8°C after opening the pack.

Washing solution

Check **Stock washing solution (№1)** for the presence of salt crystals. If crystals are seen in the solution, dissolve them by heating at 35-37 °C.

Dilute the **stock washing solution (№1)** with distilled or deionised water (see chart below) shake intensively.

Washing solution is stable for 10 days at 2-8 °C.

Number of wells	Stock washing solution (№1)	Distilled water
16	6 ml	270 ml
32	8 ml	360 ml
48	12 ml	540 ml
64	16 ml	720 ml
80	20 ml	900 ml
96	25 ml	1125 ml

Conjugate solution

Dilute in the ratio 1:50 the **conjugate** with **solution for conjugate dilution (№4)** (see chart below) in a clean vial. Mix well avoiding foaming.

Conjugate solution has to be prepared before use.

Conjugate solution is stable for 2 weeks at 2-8 °C.

Number of wells	Conjugate (concentrate 50X)	Solution for conjugate dilution (№4)
16	40 µl	2 ml
32	80 µl	4 ml
48	120 µl	6 ml
64	160 µl	8 ml
80	200 µl	10 ml
96	240 µl	12 ml

TMB substrate

To prepare **TMB substrate**, combine the required amount of **chromogen TMB** in a clean vial in equal parts with **solution for chromogen preparation (№5T)** according to the number of wells being run (see chart below). Mix well. TMB substrate is to be colourless before use.

The TMB substrate is to be kept away from light and no solution contacts with metals or metal ions are allowed.

The substrate solution is to be prepared before use.

TMB substrate is stable for 2 weeks at room temperature (18-25 °C) if kept in the dark.

Number of wells	Chromogen TMB	Solution for chromogen preparation (№5T)
16	1 ml	1 ml
32	2 ml	2 ml
48	3 ml	3 ml
64	4 ml	4 ml
80	5 ml	5 ml
96	6 ml	6 ml

Procedural notes

Authenticity of results depends on correct execution following instructions:

- Reagents should not be used beyond the expiry date shown on the package label.
- Reagents should not be mixed from different lots during performing test.
- Reagents and samples should be at room temperature (18-25°C) before testing is run. Return the reagents to 2-8°C after use.
- The temperature in room where analysis performing should be in the range 18-25°C.
- It should accurately dissolve reagents avoiding its contamination.
- Do not perform the test in the presence of reactivity vapours (for example, from sodium hypochlorite, acids, alkalis, or aldehydes) or dust because the enzymatic activity of the conjugate may be affected.
- Use glass vessels thoroughly washed and rinsed with deionized water or use disposable ones.
- Do not allow drying contents of wells on all stages of procedure.
- Enzyme reaction is sensitive to metal ions, so avoid contacting with metal elements.
- TMB substrate (solution for chromogen preparation + chromogen TMB) is to be colourless. Appearance of colouring after dilution is evidence of unavailability for using and solution is to be replaced. The solution is to be prepared in clean plastic ware or clean glassware. The reagent is to be kept in dark.